Preparation and Characterization of Porous Poly(vinyl ester) Resin Monoliths as Separation Media

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ABSTRACT: Porous poly(vinyl ester) resin monolithic supports were first prepared by radical polymerization of the continuous phase of oil in water high-internal-phase emulsions. Vinyl ester (VE) resin was used as the monomer, ethylene glycol dimethacrylate was used as a crosslinker, and poloxamer 127 was used as the emulsifier in the emulsion polymerization. The prepared columns were evaluated by scanning electron microscopy, mercury intrusion porosimetry, and Fourier transform infrared spectroscopy to observe the morphological characteristics and confirm the absorbance based on the VE resin polymer. The obtained monolith showed not only higher column permeability but also lower back pressure and higher column efficiency. To investigate the absorption performance of the monolithic column, a maximum loading capacity experiment was also applied with lysozyme (Lys), and the results show that the maximum adsorption of the poly(vinyl ester) resin monolith was 1.579 mg/g. Moreover, the capabilities of separation on this column in conjunction with high-performance liquid chromatography were investigated. Immunoglobulin could be separated from human plasma and chicken egg yolk with high resolution within 4 min. Additionally, fast separation of two mode proteins (interleukin-18 and Lys) was achieved on the monolith within 2 min at the rate of 1445 cm/h, which demonstrated the potential of the poly(vinyl ester) resin monolith for the fast separation of proteins. J Appl Polym Sci 119: 412–418, 2011

Key words: chromatography; emulsion polymerization; proteins

INTRODUCTION

Monolithic materials nowadays hold an impressively strong position in separation science. Polymer monolithic columns have been applied in a variety of shapes and separation modes for the purification of large molecules, such as proteins, polynucleotides, and even viruses.[1–5] Until now, the usual method for preparing monoliths has been polymerization in the presence of porogenic solvents. Because of the fast phase separation between growing polymer chains and porogenic solvents, the resulting monoliths consist of mesoporous microglobules aggregated in a cluster.[6] The inherent disadvantages are the adverse effects, such as a limited pore surface area for molecular recognition sites, larger eddy diffusion through irregular interstitial channels, and low permeability.[7] These limitations lead to peak broadening and, finally, decrease the column efficiency.[8,9] Therefore, the determination of how to prepare a highly porous monolith to eliminate eddy dispersion and permit high-resolution separations with short flow paths is of growing concern.

An alternative method for the preparation of highly porous monolithic material is the polymerization of a continuous phase with a high-internal-phase emulsion (HIPE). Typically, the polymer has an open cellular structure. The internal phase is trapped inside the continuous phase during the polymerization. After the internal phase is removed, the open cellular structure with interconnects are obtained. Such monolithic polymers (Poly-HIPEs)[10,11] have been initially prepared as styrene/divinylbenzene copolymers and applied as forerunners in biological tissue scaffolds,[12–15] as catalysis supports,[16,17] and as supports for filtration.[18] An attractive feature of monoliths as chromatographic support materials is that no frits are required because the monolith rods are directly synthesized within the column. Another characteristic is that the pore size distribution (macropore, mesopore, and micropore) can be easily controlled by the manipulation of the reaction conditions.[19,20] Moreover, the porous structures of the monolithic columns lead to low flow resistance and high rates of mass transfer.[19–21] The convective mass transfer between the mobile phase and liquid phase allow chromatographic performance

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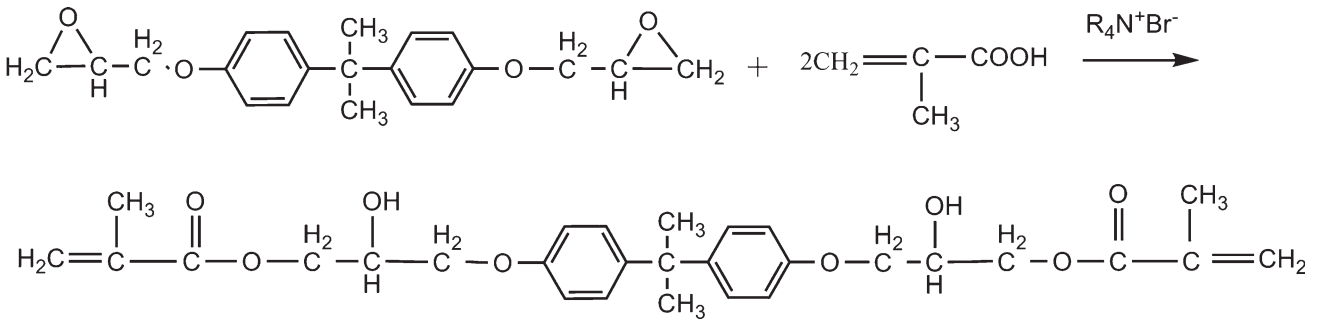

Figure 1 Synthesis process of the VE resin.

at a very fast velocity without a loss in resolution. Yao[22] prepared a series of glycidyl methacrylate-based polymer monoliths, including spherical walled structures, submicrometer skeletons, and lamellar morphologies, which were reproducibly prepared by HIPE in combination with block copolymer chemistry.

The open cellular structure of Poly-HIPE monolithic materials suggests the possible applications of such monoliths as separation media. We were, therefore, intrigued by the possibility of preparing vinyl ester (VE) resins based on Poly-HIPE monoliths. VE resins are the addition product of an epoxy resin and an unsaturated carboxylic acid. These resins have superior properties relative to unsaturated polyester systems and are inexpensive and easier to process than epoxy systems.[23] Furthermore, resins with much higher thermal properties, good mechanical capacities, and alkali resistance were obtained.[24]

In this study, a novel poly(vinyl ester resin-*co*-ethylene glycol dimethacrylate) Poly-HIPE monolithic material was first prepared. Its characterization and application as a chromatographic support without any modification for the fast separation of proteins are described.

EXPERIMENTAL

Materials

VE resin was synthesized from bisphenol A diglycidyl ether. Ethylene glycol dimethacrylate was purchased from Acros (NJ), and poloxamer 127 (PF127) was provided by Chuangqi (Beijing, China). Both were used without additional purification. Potassium persulfate and anhydrous calcium chloride were obtained from Tianjin Huadong Chemical Co. (Tianjin, China). Immunoglobulin, lysozyme (Lys), and interleukin-18 (IL-18) were bought from Sigma Chemical Co. (St Louis, MO). Eggs were purchased from a common supermarket. Human plasma was provided by volunteers. The samples were prepared by the dissolution of the appropriate proteins in phosphate buffer with a concentration of 0.001 mol/L.

Preparation of the VE resin

VE resin was prepared according to a procedure described previously.[25,26] Bisphenol A diglycidyl ether (10 g), tetrabutylammonium bromide (0.2 g), and 1,4-dioxane (10 mL) were stirred together in a 50-mL, round-bottom flask and heated to 90°C; then, 4.3 mL of methacrylic acid was slowly introduced into the mixture. The process is shown in Figure 1.

Preparation of HIPE

The reactor was charged with 0.5 g of monomer (VE) resin, 0.6 g of crosslinker (ethylene glycol dimethacrylate), and 0.09 g of PF127 and stirred with a muddler (polytetrafluoroethylene) at 400 rpm. Then, the aqueous phase, consisting of 2.72 mL of initiator (potassium persulfate, 0.2% w/v based on H_2O) and 0.68 mL of electrolyte (anhydrous calcium chloride, 1% w/v based on H_2O), was added dropwise. Stirring was continued for 30 min until a white, milky emulsion was formed. The stability of HIPE was evaluated by the percentage of the separation weight. The lower the value was, the more stable the HIPE was.[27]

Preparation of the poly(vinyl ester) resin monolith

The poly(vinyl ester) resin was prepared as follows: The emulsion was purged with nitrogen with a stainless steel needle through a silicone resin septum for 5 min, then transferred to a mold (stainless steel column, 50 mm × 4.6 mm *i.d.*), and cured at 55°C for 36 h. After it was cooled to room temperature, the column was connected to a high-performance liquid chromatography (HPLC) system to remove templated polymers and other soluble compounds with deionized water and methanol.

Fourier transform infrared spectroscopy (FTIR-8400S; Shimadzu, Tokyo, Japan) showed the characteristic peaks of the groups in the poly(vinyl ester) resin as follows. The broad band at 3400 cm^{-1} was the —OH group; the spectrum at 3000–2900 cm^{-1} was due to the stretching in C—H of an aromatic group or alkenes. The clear adsorption peak at 1725 cm^{-1} was due to the C=O of esters; the spectra at

1510 and 1100 cm^{-1} showed the presence of the phenyl ring vibration and C—O—C group, respectively.

Loading of the hydroxyl groups on the monolith

The excess of acetic acid after the acetylation of the resin with acetic anhydride was titrated to determine the average loading of the hydroxyl groups on the monolithic column.[28] Monolithic material (150 mg) was placed in a 100-mL flask along with 1.0 mL of acetic anhydride and 5.0 mL of pyridine. The mixture was stirred at 60°C for 12 h. At the end of the acetylation, 10 mL of water was added to the flask to convert excess acetic anhydride into the corresponding acetic acid, which was titrated with a 1.0 mol/L NaOH aqueous solution (a phenolphthalein solution was used as the indicator) at room temperature. Blank titration was performed in the same way to prevent systematic errors. The amount of hydroxyl groups on the monolithic column was taken as the average of three parallel experiments.

Column efficiency of the monolith

The plate theory was used to investigate the efficiency of the monolith, and the height of the equivalent theoretical plate (HETP) was determined by the following equation:[29]

$$\mathrm{HETP} = \frac{L\sigma^2}{\mu_1^2}$$

where L is the length of the column (mm), σ^2 is the peak variance, and μ_1 is the first absolute moment. Bovine serum albumin (BSA; 2 mg/mL in the mobile phase) was used as a tracer in the experiment under unretained conditions. The mobile phase was 0.3 mol/L NaCl in 10 mmol/L Tris-HCl buffer (pH 7.6). After the column was equilibrated, 20 μL of BSA solution was injected. The dead volume of the system was measured by the injection of acetone solution via blank experiments. From the chromatogram, σ^2 and μ_1 were calculated, respectively, from

$$\sigma^2 = \frac{\int t^2 c(t)dt}{\int c(t)dt}$$

$$\mu_1 = \frac{\int tc(t)dt}{\int c(t)dt}$$

where t is the time (s) and $c(t)$ is the BSA concentration at the column exit (mg/mL) as a function of time.

Chromatographic measurements

HPLC measurement was carried out with an 1100 system from Agilent Technologies (Agilent, USA). The HPLC system consisted of a quaternary pump with an online vacuum degasser, an autosampler with variable injection capacity from 0.1 to 100 μL, and a UV detector. Chromatographic separation was achieved on a polymeric monolithic column (50 mm × 4.6 mm *i.d.*). All sample solutions injected in the chromatographic system were filtered through a Millipore membrane (0.45 μm) to remove particles and large aggregates. The flow rate was 1.0 mL/min, the detection was performed at 280 nm, and the temperature was 25°C.

RESULTS AND DISCUSSION

Preparation of the HIPE and poly(vinyl ester) resin monolith

The synthesized VE resin was used as a monomer to prepare the HIPE with potassium persulfate as an initiator. The emulsion was influenced by the content of PF127. At a lower mass of PF127 (<0.02 g), two distinct phases appeared in a few minutes: a transparent upper dispersed phase and a white, milky lower organic phase. In addition, when its content exceeded 0.15 g, the emulsions showed a much higher viscosity, and it was difficult to form interconnected monolithic material. Meanwhile, the influence of the crosslinker, its content, and the amount of electrolyte were investigated. When the amount of crosslinker or electrolyte changed, the structure of the emulsion changed a little, too. However, the monolithic material became harder. When the amount of crosslinker or electrolyte was too large, the emulsion prepared was not suitable to form the HPLC stationary phase.

The stability of the emulsions was determined with a certain amount of emulsion being centrifuged and put at a constant temperature for 24 h. The results showed that the percentage of the separation weight was 3.89%.

Structural characterization and loading of hydroxyl groups on the monolith

The prepared columns were evaluated by scanning electron microscopy (Miniscope, Hitachi Co., Tokyo, Japan) to observe the morphological characteristics based on the VE resin polymer (Fig. 2). Each large pore of the VE resin Poly-HIPE monolith was surrounded by a wall containing small pores. Moreover, the total pore area, average pore diameter, and porosity measured by mercury intrusion porosimetry were 121.96 m^2/g, 0.85 μm, and 75.76%, respectively. These results indicate that small-sized

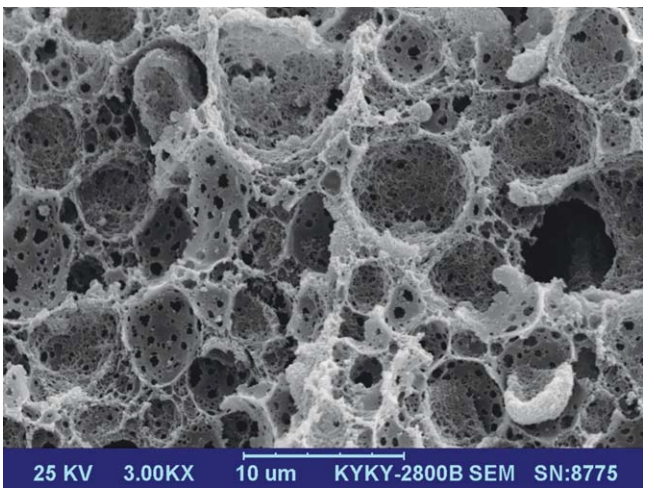

Figure 2 Scanning electron micrograph of the poly(vinyl ester) resin monolith. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

skeletons provided larger surface areas and, thus, excellent accessibility of the reactive sites.

Furthermore, the amount of hydroxyl groups on the monolithic materials was determined. The resins were acetylated by reaction with an excess of acetic anhydride, which was then converted to acetic acid and titrated with a base. The results show that the average loading of hydroxyl groups was 14.0 mmol/g.

Linear velocity versus back-pressure drop

To determine the mechanical stability and high-throughput elution of the poly(vinyl ester) resin monolith, the back-pressure drops at several linear velocities were investigated. Figure 3 shows the effect of the flow rate on the back pressure with water and methanol as the mobile phase. Actually, the pressure drops were 0.8 and 1.0 MPa at 361 cm/h. A better linear dependence of the column pressure on the flow rate was apparent from a regression factor (r) of no less than 0.999. These results clearly show that the poly(vinyl ester) resin monolith was suitable for high-throughput elution.

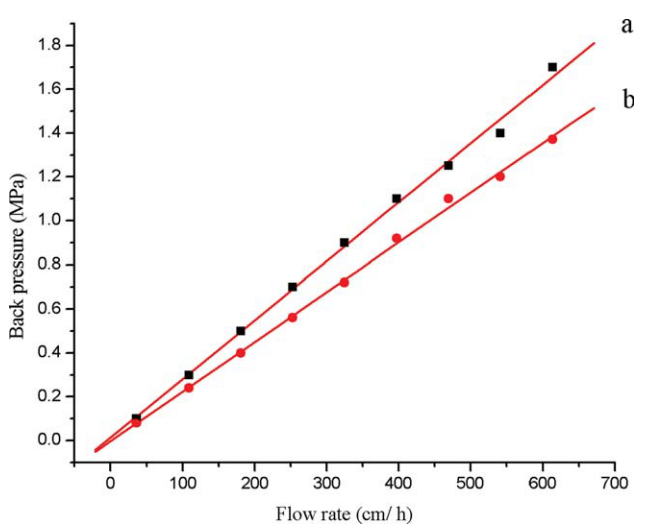

Figure 3 Linear velocity and back-pressure drop on the poly(vinyl ester) resin monolith: (a) methanol and (b) deionized water (column size: 50 mm × 4.6 mm *i.d.*, mobile phase: H_2O). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

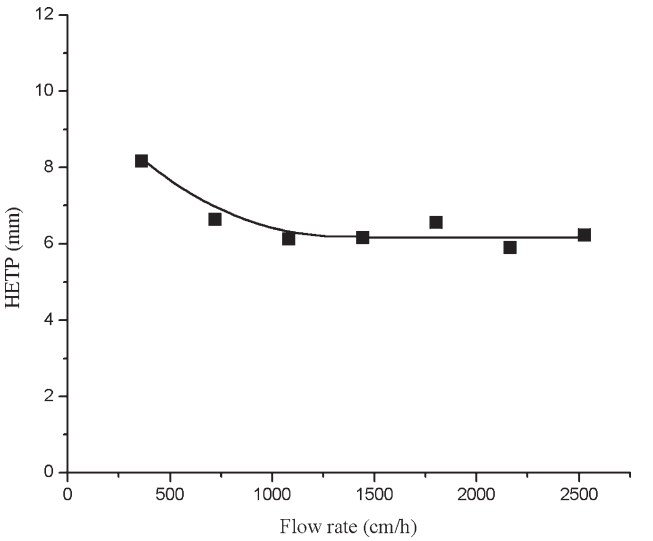

Figure 4 Dependence of the column efficiency on the mobile-phase flow rate for the poly(vinyl ester) resin monolith (column size: 50 mm × 4.6 mm *i.d.*, mobile phase: 0.3 mol/L NaCl in 10 mmol/L Tris-HCl buffer at pH 7.6, feed: 2 mg/mL BSA in buffer, injection size = 20 μL).

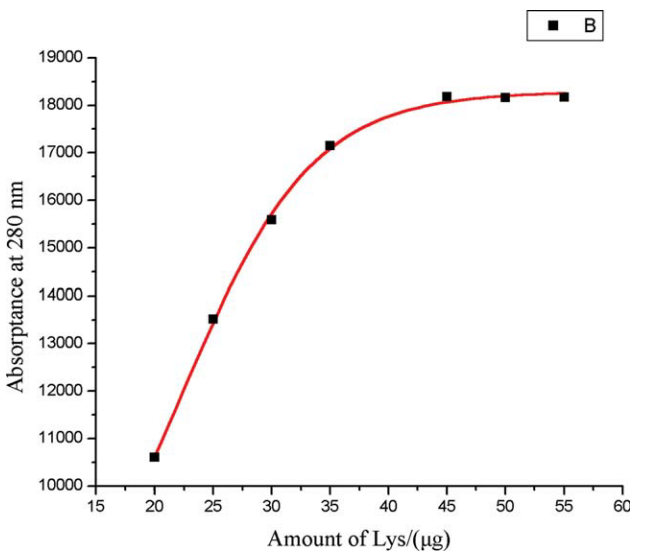

Figure 5 Peak area as a function of the loading of Lys to the poly(vinyl ester) resin monolith [column size: 50 mm × 4.6 mm *i.d.*, gradients: 0–35 min and 100% A (H_2O) and 35.01–50 min and 100% B (1.0 mol/L NaCl)]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

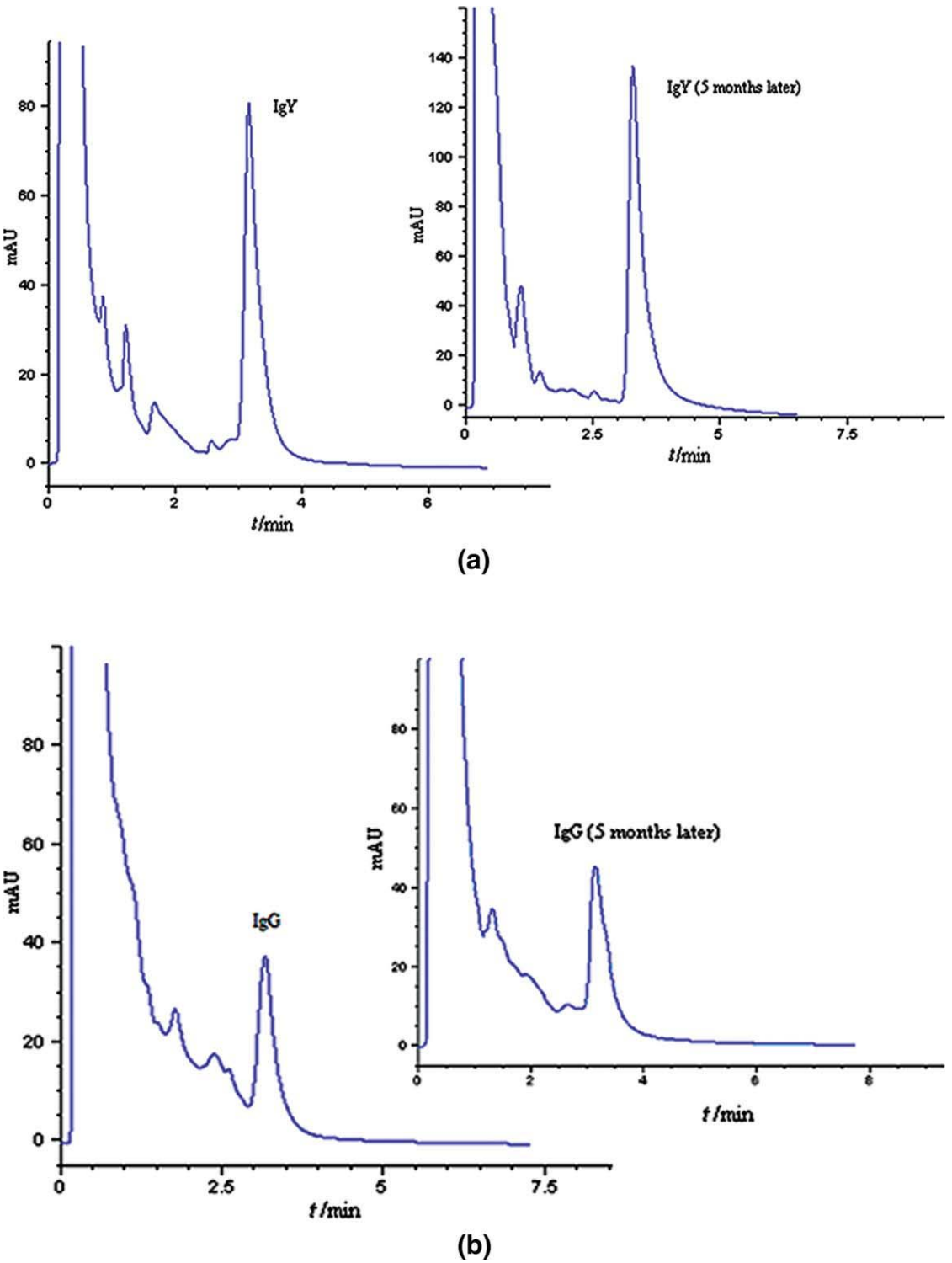

Figure 6 Chromatographic testing of the poly(vinyl ester) resin monolith: chromatograms of separation of (a) IgY from egg yolk and (b) IgG from human plasma [column size: 50 mm × 4.6 mm *i.d.*, gradients: 0–2 min and 100% A (H_2O) and 2.01–5 min from 30 to 100% B (1.0 mol/L NaCl), injection size = 1 μL]. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Column efficiency

HETP is a typical criterion to used describe the overall column efficiency. In this study, BSA was used as a probe solute, and HETP was measured under the nonretained conditions. Figure 4 shows the relationship between HETP and the flow velocity for the poly(vinyl ester) resin monolith. The result show that the values of HETP decreased and remained approximately unchanged from 1000 to 2520 cm/h.

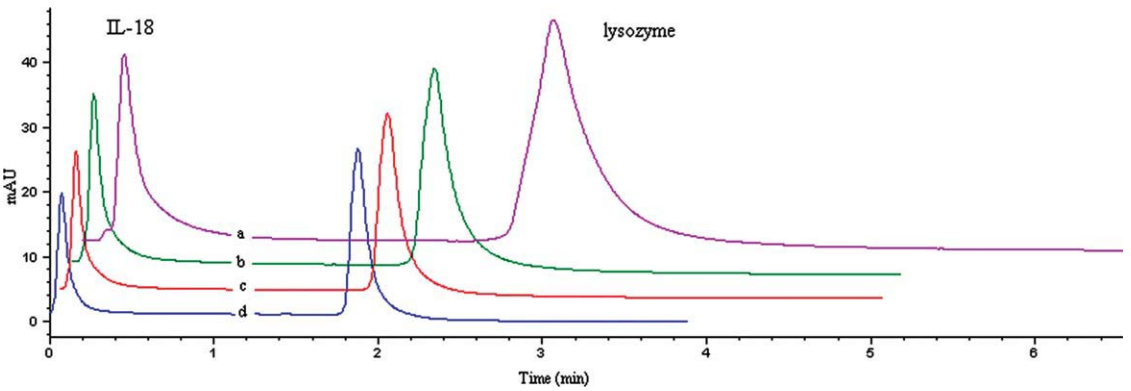

Figure 7 Fast separation of IL-18 and Lys on the poly(vinyl ester) resin monolith [column size: 50 mm × 4.6 mm *i.d.*, gradients: 0–1.5 min and 100% A (0.001 mol/L phosphate buffer at pH 7.8) and 1.51–2.5 min from 30 to 100% B (in which 1 mol/L NaCl was added to buffer A)]. Gradient elution was at flow rates of (a) 361, (b) 722, (c) 1083, and (d) 1445 cm/h (injection size = 5 μL). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The column efficiency increased with the flow rate at a low flow rate range (200 cm/h). This could be explained by the nature of the inner macroporous structures in the monolith. With increasing mobile-phase velocity, more superpores in the monolith were expected to be involved in the convective mass transport. The diffusion resistance did not play a significant role in the column efficiency. These results demonstrate that the poly(vinyl ester) resin monolith also provided rapid mass transfer in the separation.

Dynamic capacity of Lys on the polymeric monolithic column

A maximum loading capacity experiment was done to investigate the absorption performance of the monolithic column. Different amounts of Lys were injected into the monolithic column when water was used as the mobile phase. Then, the column was eluted by 1 mol/L NaCl. Because the amount of adsorption was fixed for a given column, the peak area increased with increasing injection amount before the column reached saturation. Even more Lys was injected into the column, only the part that was adsorbed on the column when the column reached saturation. So the peak area remained unchanged when the injection amount increased. The result is shown in Figure 5. The maximum adsorptive of Lys was 1.579 mg/g.

Chromatographic testing of the monolithic column

The chromatographic possibilities of the poly(vinyl ester) resin monolith were investigated. Immunoglobulin Y (IgY) was separated from egg yolk within 4 min with high resolution, as shown in Figure 6(a). Many distinct peaks were obtained in the chromatogram. Meanwhile, immunoglobulin G (IgG) also could be separated from human plasma in a short time by the monolith [Fig. 6(b)]. From the peak shape, we concluded that the structure of the monolith without any modification was suitable for chromatographic separations. Furthermore, the separation performances of the column after 5 months are indicated in Figure 6(a,b). There was no significant difference in the retention times or resolution. These results strongly suggest the further potential of this novel monolith for the efficient downstream processing of biomolecules.

Figure 7 shows the separation of a two-mode protein mixture (IL-18 and Lys) on the poly(vinyl ester) resin monolith with gradient elution at different flow rates. The baseline separation of the two proteins could be achieved at a flow rate of 361 cm/h within 3 min, but the chromatographic profile was almost the same as that of 1445 cm/h. Because of the much lower pressure drop, separations of proteins on the column could be performed with a high flow rate to save operation time; this showed the potential of the poly(-vinyl ester) resin monolith for the rapid analysis and separation of proteins. Furthermore, the column revealed good stability in the experiments. The pressure drop and column efficiency did not change with the accumulation of more than 100 injections.

Reproducibility

The poly(vinyl ester) resin monolith was evaluated for run-to-run, day-to-day, and column-to-column

TABLE I
Reproducibility of the Retention Time for the Poly(vinyl ester) Resin Monolith (RSD %)

	Run to run ($n = 6$)	Day to day ($n = 3$)	Column to column ($n = 3$)
IL-18	1.6	3.7	2.1
Lys	1.8	4.3	1.9
IgY	2.7	3.9	2.1
IgG	2.9	4.5	3.0

n, the number of sample introduction.

reproducibility. The relative standard deviations (RSDs) for the retention time reproducibility of the columns are shown in Table I. The retention time reproducibility of the column was less than 2.9% RSD from run to run, the day-to-day reproducibility was less than 4.5% RSD, and the column-to-column reproducibility was less than 3.0% RSD. These low values suggest that this monolith was very reproducible.

CONCLUSIONS

In this study, the emulsion polymerization of VE resin showed good prospects as an alternative method for the preparation of highly porous monolithic supports for separation. The monoliths combined high porosity, good mechanical properties, and hydroxyl groups in the polymer matrix to offer the possibility of chemical modifications. Moreover, accelerated mass transfer induced by the convective flow of the mobile phase in the medium was also demonstrated by its low column back pressure, high column efficiency, and high protein resolution at a high flow velocity. These results suggest further potential of this novel monolith for the efficient downstream processing of biomolecules. In future studies, we hope to optimize the pore size, pore homogeneity, physical strength, and chemical characterization by modification of the basic polymer and preparation conditions with a view to developing practical separation media.

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